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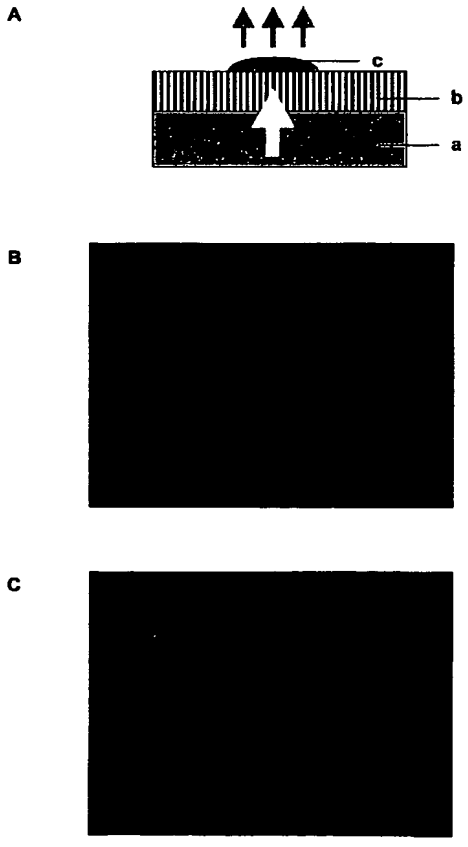
(54) Title: HIGH THROUGHPUT CELLULAR RESPONSE ASSAY USING MICROARRAYS



(57) Abstract: The present invention relates to methods for screening of cellular responses of cellular components comprising:(a) providing cellular components on the surface of a substrate, said substrate having immobilized thereon an array of detector molecules;(b) delivering test compounds to positions on the substrate corresponding to the arrayed detector molecules on the surface of said solid substrate;(c) incubating said test compounds with said cellular components on the surface of the solid support, under conditions allowing the induction of cellular responses;(d) assaying said cellular responses; and,identifying and characterizing the cellular responses induced by said test compounds. The present invention further relates to the uses of said methods as well as microarrays and kits for carrying out said methods.

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AMENDED CLAIMS
[received by the International Bureau on 9 January 2004 (09.01.04);
original claims 1-35 replaced by amended claims 1-34 (5 pages)]

Claims

1. A method for screening of cellular responses of cellular components comprising:
   (a) providing cellular components on the surface of a solid porous substrate, wherein
      (i) said solid porous substrate retains said cellular components on its surface, and wherein,
      (ii) said solid porous substrate has immobilized therein, within the pores, an array of detector molecules;
   (b) delivering test compounds to positions on the substrate corresponding to the arrayed detector molecules on the surface of said solid porous substrate;
   (c) incubating said test compounds with said cellular components on the surface of the solid porous substrate, under conditions allowing the induction of cellular responses;
   (d) assaying said cellular responses; and,
   (e) identifying and characterizing the cellular responses induced by said test compounds.

2. The method according to claim 1, wherein said solid substrate is a flow-through porous solid substrate.

3. The method according to any of claims 1 to 2, wherein said providing of cellular components on the surface of a substrate is by a deposit directly on said substrate of an inoculum or a culture.

4. The method according to any of claims 1 to 3, wherein said delivering of test compounds is by means of contact force.

5. The method according to claim 4, wherein said contact force is a capillary force or a piezo-electric force.

6. The method according to any of claims 1 to 5, wherein the nutrient(s) are provided from underneath the pores of the solid surface.

7. The method according to any of claims 1 to 6, wherein said assaying of cellular responses is by:
   (a) providing a detection agent to the cellular components;
   (b) washing off excess of unincorporated detecting agent;
   (c) detecting the presence or absence of a change in detectable signal, the presence of a change in detectable signal indicating a cellular response.

8. The method according to any of claims 1 to 7, wherein said cellular response is assayed in whole broth or cell culture medium, in isolated cells such as pelleted cells, in supernatant of the cellular components, or in lysate of the cellular components.

9. The method according to any of claims 1 to 8, wherein said delivery of test compounds is by a means chosen from the group comprising a delivery mask, a high precision x-y-z pipettor, inkjet printer, and manual handling.

10. The method according to claim 9, wherein said delivery of test compounds is by means of a high precision x-y-z pipettor or inkjet printer.

11. The method according to any of claims 1 to 10, wherein said identifying of the cellular responses is by luminescence.

12. The method according to claim 11, wherein said luminescence is fluorescence.

13. The method according to any of claims 1 to 12, wherein said cellular components are selected from the group comprising mammalian cells, insect cells, yeast cells, fungal cells plant cells and microbial cells including bacterial cells, including cellular vesicles, cellular organelles, tissue sections, and whole organisms including nematodes.

14. The method according to any of claims 1 to 13, wherein said detector molecules are selected from the group comprising nucleic acids including modified analogues

thereof, peptides, proteins, and antibodies including antibody fragments, enzyme substrates and specific dyes.

15. The method according to any of claims 1 to 14, wherein said cellular responses are chosen from the group comprising chemically induced or physiological events in the cell including lysis, apoptosis, growth inhibition, and growth promotion; production, secretion, and surface exposure of a protein or other molecule of interest by the cell; membrane surface molecule activation including receptor activation; trans-membrane ion transports; and transcriptional regulations.

16. The method according to claim 15, wherein said molecule of interest is selected from the group comprising peptides including lipopeptides, glycosylated peptides and antimicrobial peptides, polypeptides, proteins, enzymes, antimicrobial molecules, primary and secondary metabolites, and small organic molecules including pharmaceutical molecules.

17. The method according to any of claims 1 to 16, wherein said test compound is a drug or any compound which is useful in the selection process of a drug candidate.

18. The method according to claim 17, wherein said test compound is a drug selected from a chemical or natural drug candidate library.

19. The method according to any of claims 1 to 18, wherein said solid substrate is a metallo-oxide substrate.

20. The method according to claim 19, wherein said solid substrate is an aluminum-oxide substrate.

21. The method according to any of claims 1 to 20, wherein said assaying is performed in real-time.

22. The method according to any of claims 1 to 21, wherein said assaying is an end-point assaying.

23. The method according to claim 7, wherein said providing a detection agent to the cellular components occurs prior to delivering of test compound thereby providing pre-labeled cellular components.

24. Use of a method according to any of claims 1 to 23, for monitoring induced cellular responses of host cells.

25. Use of a method according to any of claims 1 to 23, for on-chip recombination, transformation or viral introduction of cellular components.

26. Use of a method according to any of claims 1 to 23, for functional screening of cellular responses upon assaying host cells with test compounds.

27. A microarray for performing a method according any of claims 1 to 23, wherein an array of test compounds is provided within predefined regions, said test compounds are in liquid solution and not immobilized in the substrate.

28. A microarray for performing a method according any of claims 1 to 23, wherein an array of cellular components is provided in predefined regions on a substrate, said cellular components being conditioned for preservation on said substrate.

29. A microarray for performing a method according any of claims 1 to 23, wherein a cellular component is provided on a substrate, said cellular component being conditioned for preservation on said substrate.

30. The microarray according to any of claims 27 to 29, wherein an array of detector molecules is immobilized within the substrate.

31. The microarray according to claim 30, wherein said array of detector molecules comprises a plurality of equal detector molecules or a plurality of different detector molecules.

32. The microarray according to claim 28 or 29, wherein said condition is chosen from the group comprising lyophilization and glycerol dissolution.

33. Use of a microarray according to any of claims 27 to 32, for providing cellular components on the surface of a substrate for use in a method according to any of claims 1 to 23, thereby providing said cellular components with low spreading properties.

34. A kit for performing a method according to any of claims 1 to 23, comprising a microarray according to any of claims 27 to 32.

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      (ii) said solid porous substrate has immobilized therein, within the pores, an array of detector molecules;
   (b) delivering test compounds to positions on the substrate corresponding to the arrayed detector molecules on the surface of said solid porous substrate;
   (c) incubating said test compounds with said cellular components on the surface of the solid porous substrate, under conditions allowing the induction of cellular responses;
   (d) assaying said cellular responses; and,
   (e) identifying and characterizing the cellular responses induced by said test compounds.

2. The method according to claim 1, wherein said solid substrate is a flow-through porous solid substrate.

3. The method according to any of claims 1 to 2, wherein said providing of cellular components on the surface of a substrate is by a deposit directly on said substrate of an inoculum or a culture.

4. The method according to any of claims 1 to 3, wherein said delivering of test compounds is by means of contact force.

5. The method according to claim 4, wherein said contact force is a capillary force or a piezo-electric force.

6. The method according to any of claims 1 to 5, wherein the nutrient(s) are provided from underneath the pores of the solid surface.

7. The method according to any of claims 1 to 6, wherein said assaying of cellular responses is by:
   (a) providing a detection agent to the cellular components;
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   (c) detecting the presence or absence of a change in detectable signal, the presence of a change in detectable signal indicating a cellular response.

8. The method according to any of claims 1 to 7, wherein said cellular response is assayed in whole broth or cell culture medium, in isolated cells such as pelleted cells, in supernatant of the cellular components, or in lysate of the cellular components.

9. The method according to any of claims 1 to 8, wherein said delivery of test compounds is by a means chosen from the group comprising a delivery mask, a high precision x-y-z pipettor, inkjet printer, and manual handling.

10. The method according to claim 9, wherein said delivery of test compounds is by means of a high precision x-y-z pipettor or inkjet printer.

11. The method according to any of claims 1 to 10, wherein said identifying of the cellular responses is by luminescence.

12. The method according to claim 11, wherein said luminescence is fluorescence.

13. The method according to any of claims 1 to 12, wherein said cellular components are selected from the group comprising mammalian cells, insect cells, yeast cells, fungal cells plant cells and microbial cells including bacterial cells, including cellular vesicles, cellular organelles, tissue sections, and whole organisms including nematodes.

14. The method according to any of claims 1 to 13, wherein said detector molecules are selected from the group comprising nucleic acids including modified analogues

thereof, peptides, proteins, and antibodies including antibody fragments, enzyme substrates and specific dyes.

15. The method according to any of claims 1 to 14, wherein said cellular responses are chosen from the group comprising chemically induced or physiological events in the cell including lysis, apoptosis, growth inhibition, and growth promotion; production, secretion, and surface exposure of a protein or other molecule of interest by the cell; membrane surface molecule activation including receptor activation; trans-membrane ion transports; and transcriptional regulations.

16. The method according to claim 15, wherein said molecule of interest is selected from the group comprising peptides including lipopeptides, glycosylated peptides and antimicrobial peptides, polypeptides, proteins, enzymes, antimicrobial molecules, primary and secondary metabolites, and small organic molecules including pharmaceutical molecules.

17. The method according to any of claims 1 to 16, wherein said test compound is a drug or any compound which is useful in the selection process of a drug candidate.

18. The method according to claim 17, wherein said test compound is a drug selected from a chemical or natural drug candidate library.

19. The method according to any of claims 1 to 18, wherein said solid substrate is a metallo-oxide substrate.

20. The method according to claim 19, wherein said solid substrate is an aluminum-oxide substrate.

21. The method according to any of claims 1 to 20, wherein said assaying is performed in real-time.

22. The method according to any of claims 1 to 21, wherein said assaying is an end-point assaying.

23. The method according to claim 7, wherein said providing a detection agent to the cellular components occurs prior to delivering of test compound thereby providing pre-labeled cellular components.

24. Use of a method according to any of claims 1 to 23, for monitoring induced cellular responses of host cells.

25. Use of a method according to any of claims 1 to 23, for on-chip recombination, transformation or viral introduction of cellular components.

26. Use of a method according to any of claims 1 to 23, for functional screening of cellular responses upon assaying host cells with test compounds.

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31. The microarray according to claim 30, wherein said array of detector molecules comprises a plurality of equal detector molecules or a plurality of different detector molecules.

32. The microarray according to claim 28 or 29, wherein said condition is chosen from the group comprising lyophilization and glycerol dissolution.

33. Use of a microarray according to any of claims 27 to 32, for providing cellular components on the surface of a substrate for use in a method according to any of claims 1 to 23, thereby providing said cellular components with low spreading properties.

34. A kit for performing a method according to any of claims 1 to 23, comprising a microarray according to any of claims 27 to 32.